

# UNCLASSIFIED

AD NUMBER
AD850070
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; MAR 1969. Other requests shall be referred to Department of the Army, Fort Detrick, Attn: Technical Release Branch/TIO, Frederick, MD 21701.
AUTHORITY
Biological Defense Research Lab ltr dtd 29 Sep 1971

THIS PAGE IS UNCLASSIFIED

AD

TECHNICAL MANUSCRIPT 520

A SURVEY OF GENOMIC MAPS  
IN STRAINS OF STAPHYLOCOCCUS AUREUS

AD850070

Robert A. Altenbern

MARCH 1969

DEPARTMENT OF THE ARMY  
Fort Detrick  
Frederick, Maryland

STATEMENT #2 UNCLASSIFIED  
This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Dept. of Army, Fort Detrick, ATTN: Technical Release Branch/TID, Frederick, Maryland 21701

D D C  
RECEIVED  
APR 11 1969  
C

DISPOSITION BY		
WHITE SECTION	<input type="checkbox"/>	
DIFF SECTION	<input checked="" type="checkbox"/>	
UNCLASSIFIED	<input type="checkbox"/>	
JUSTIFIED ON		
BY		
DISTRIBUTION/AVAILABILITY CODES		
DIST.	AVAIL.	SPECIAL
2		

Reproduction of this publication in whole or in part is prohibited except with permission of the Commanding Officer, Fort Detrick, ATTN: Technical Releases Branch, Technical Information Division, Fort Detrick, Frederick, Maryland, 21701. However, DDC is authorized to reproduce the publication for United States Government purposes.

#### DDC AVAILABILITY NOTICES

Qualified requesters may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

#### DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

DEPARTMENT OF THE ARMY  
Fort Detrick  
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 520

A SURVEY OF GENOMIC MAPS IN STRAINS OF  
STAPHYLOCOCCUS AUREUS

Robert A. Altenbern

Medical Bacteriology Division  
BIOLOGICAL SCIENCES LABORATORIES

Project 1B061102B71A

March 1969

# ABSTRACT

Six randomly selected strains of Staphylococcus aureus all exhibited the same gene order along the chromosome when mapped by the synchronous chromosomal replication procedure. Strains possessing multiple antibiotic resistance yielded a genomic map with all genes shifted toward significantly later replication times than in strains not bearing multiple antibiotic resistance. It appears that there is a unique site on the S. aureus chromosome for the initiation of replication.

## I. INTRODUCTION\*

The recent development of a method for mapping the chromosome of Staphylococcus aureus (the synchronous chromosomal replication method) has revealed the location of a number of genes on the genome of a single strain of this organism.<sup>1,2</sup> The voluminous literature in the past decade on gene order in various strains of Escherichia coli has shown that all these strains exhibit generally the same genomic map. In the studies reported herein, a number of strains of S. aureus were examined by the mapping method referred to above. All strains of S. aureus so far tested have yielded identical gene orders, a fact that suggests that the gene order and the positions of the genes on the chromosome describe a sequence of genetic information unique for this organism.

## II. MATERIALS AND METHODS

The method for mapping genes was essentially that previously described.<sup>3</sup> Briefly, a culture of S. aureus in Albimi Brucella broth\*\* was grown on a shaker at 37 C to late log phase. Phenethyl alcohol was added to a final concentration of 0.40% and the culture was then incubated at 30 C for 2 hours without shaking, which allows all chromosomal replication to proceed to completion but prevents initiation of another round of replication. Subsequently, 40 ml of the culture were centrifuged and the packed cells were resuspended in 100 ml of one-half strength Brucella broth. This suspension was then incubated at 30 C without shaking. Immediately after resuspension, and at 5- or 10-minute intervals thereafter, 5-ml samples were withdrawn and immediately centrifuged. The sedimented cells were rapidly resuspended in 5 ml of 0.85% saline containing 200 µg of N-methyl-N'-nitro-N-nitrosoguanidine (NG) per ml and incubated for 20 minutes at 30 C to induce mutations. At the end of the 20-minute exposure to the mutagen (NG), the suspension was either diluted and plated on minimal agar to detect mutations from auxotrophy to prototrophy or was centrifuged and resuspended in full-strength Albimi broth. Subsequent incubation of this culture on the shaker at 37 C for 5 hours allowed complete expression of recessive mutations to antibiotic resistance. Such cultures were then diluted and plated on Trypticase soy agar (BBL) containing an appropriate concentration of antibiotic.

---

\* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the author to ascertain when and where it may appear in citable form.

\*\* Albimi Laboratories, Flushing, New York.

After incubation for 48 hours at 37 C, prototrophic or antibiotic-resistant colonies were counted, and the total colony counts on triplicate plates of the same dilution were plotted against the time of sampling. The abrupt rise in total number of mutants was designated as the gene duplication time of the locus in question. The total viable count remained constant throughout the entire mapping procedure. Accurate enumeration of mutants resistant to acriflavine or to nitrofurazone requires careful selection of the proper dilution to be plated so that erratic counts obtained at higher or lower dilutions can be avoided.

All nutritional mutants were isolated from the parent strains by selection following exposure to NG. The strains employed were isolated from clinical material and were typical S. aureus. The details of composition of minimal agar, the concentrations of antibiotics employed in detecting resistant mutants, and a complete discussion of the mapping method have been presented previously.<sup>2</sup>

### III. RESULTS

Initial experiments were designed to detect the position (replication time) of the gene determining resistance to novobiocin in a number of strains. Of ten strains tested, the novobiocin locus replicated at 40 to 50 minutes for seven strains, but at 60 to 70 minutes for the other three strains. An example of each category is presented in Figure 1. Previous characterization of these strains had shown that the three strains for which the novobiocin locus replication time was 60 to 70 minutes exhibited multiple antibiotic resistance to sulfonamides, penicillin, streptomycin, and tetracycline. Presumably, these characteristics arise from the presence of a plasmid or multiple-resistance factor (MRF) bearing the resistance genes cited above. The other seven strains in the survey did not possess the MRF.

It was of interest to determine the locations of other genes on the chromosomes of the strains in question to detect differences in replication time that were related to the presence or absence of the MRF. The gene replication times and the resulting genomic map for strain Maybush are presented in Figure 2. To standardize the presentation of gene duplication times, the numbers of mutants at each test interval before the abrupt rise were averaged, and this average was divided into the actual mutant number at each sampling time, thus giving a constant base line of 1.0 for illustration of replication times of the genes.

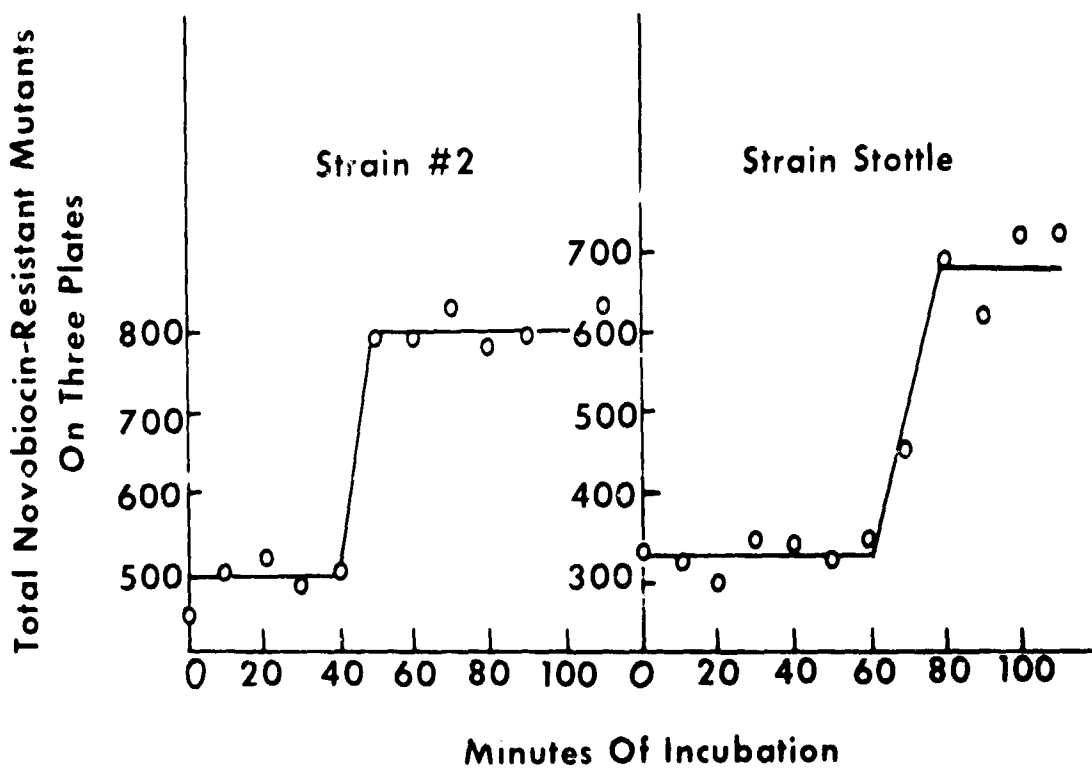


FIGURE 1. Duplication of Novobiocin Resistance Gene in *S. aureus* Strain #2, Not Bearing MRF, and Strain Stottle, Possessing MRF.

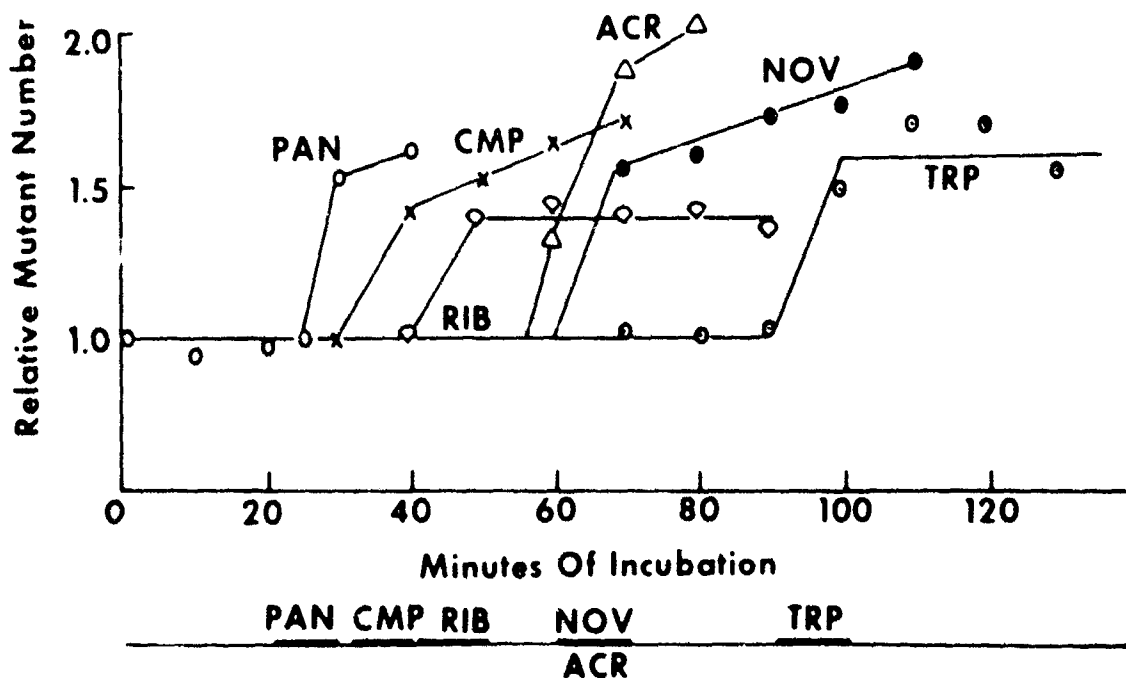


FIGURE 2. Gene Duplication Times and Genomic Map for Strain Maybush Bearing Multiple Antibiotic Resistance (MRF). ACR = acriflavin resistance; CMP = chloramphenicol resistance; PAN = pantothenate; RIB = riboflavin; NOV = novobiocin resistance; TRP = tryptophan.

Extension of this methodology to five other strains yielded the genomic maps presented in Figure 3. In this figure, the strains are grouped according to the presence and absence of the MRF. Although there are several minor anomalies in gene duplication time, it is clear that the gene replication occurs earlier in the strains without MRF than in strains harboring MRF. In addition, the consistent gene order present in all strains so far examined is apparent. Unpublished data have shown that the rate of DNA synthesis following release from phenethyl alcohol inhibition is nearly the same ( $\pm 10\%$ ) for all strains for which genomic maps have been derived (Figure 3).

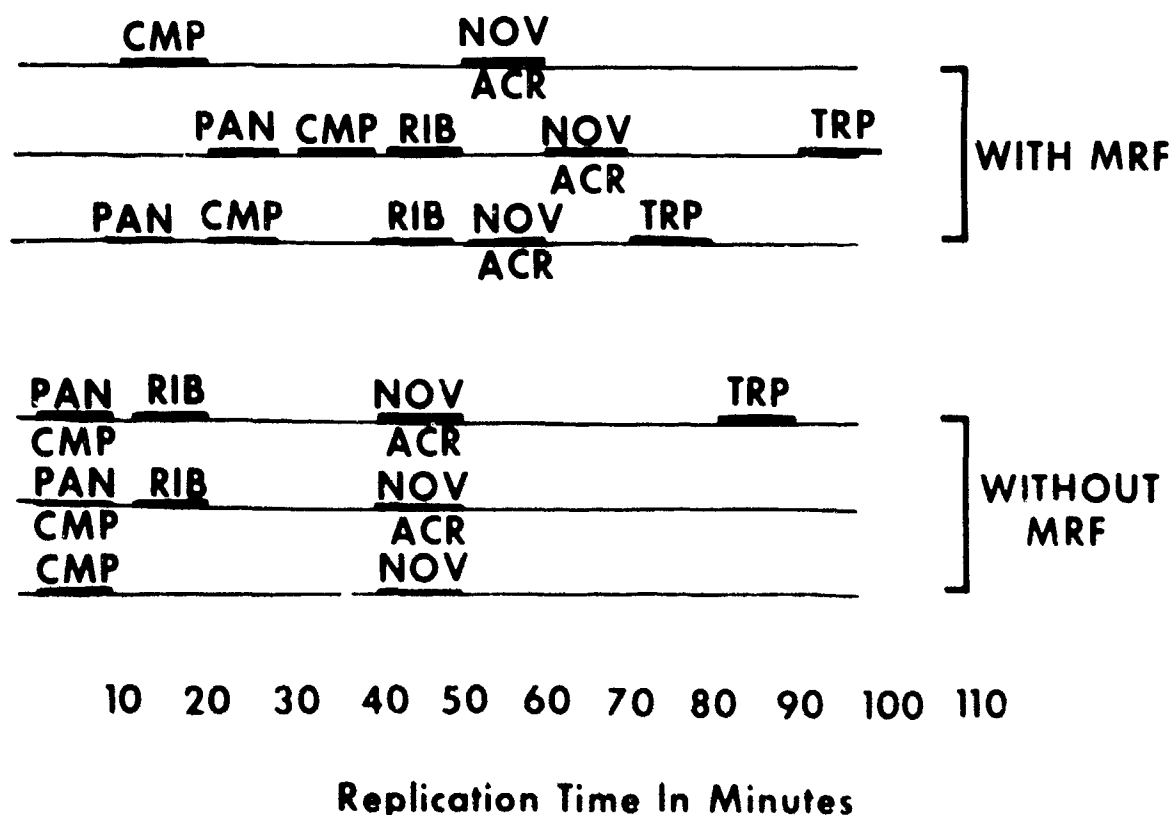


FIGURE 3. Genomic Maps of Six Strains of *S. aureus*. Gene abbreviations same as on Figure 2.

#### IV. DISCUSSION

The results presented in Figure 3 demonstrate clearly that a constant gene order occurs in at least six randomly selected strains of S. aureus. The locations of the antibiotic resistance markers were all determined with the parent types and were strictly comparable because the rates of DNA synthesis following release from phenethyl alcohol inhibition were very nearly identical. However, the location of genes governing synthesis of vitamins or tryptophan was determined with single auxotrophs that may possess differing rates of DNA synthesis and thus displace the apparent position of the gene. The time intervals between genes are also relatively constant among the various strains, thus eliminating the possibility that gross differences in rate of DNA synthesis between strains with and without MRF can account for earlier gene duplication times in strains not harboring MRF. The mechanism by which MRF delays gene duplication times is unknown, but it can be suggested that MRF duplicates first in these strains after which replication of the host genome is permitted.

The magnitude of the relative increase in mutants resulting from the abrupt rise (gene duplication time) is apparently controlled to some extent by the medium employed in the mapping procedure. An earlier paper<sup>2</sup> showed that a doubling of mutants occurred at the gene duplication time when Trypticase soy broth (BBL) was employed. In the present study, Albimi Brucella broth was used and yielded relative increases in mutant number of 1.3 to 1.5. An assessment of several other media revealed that different relative increases from 1.2 to 1.8 were obtained with different media. Albimi Brucella broth was chosen because of the sharp synchrony apparently obtained in genomic replication in this medium compared to the other media tested. Several unpublished experiments have also demonstrated that the gene duplication time of a specific locus of a single strain of S. aureus can vary as much as 10 minutes when different lots of the same medium are used. For example, the gene duplication time for novobiocin resistance was 60 to 70 minutes for strain Smith using an old lot of Albimi Brucella broth but was shortened to 50 to 60 minutes with a fresh lot of the same medium. The maps of strains bearing MRF presented in Figure 3 show some of this type of variation because one strain was studied with old Albimi Brucella broth but the other two were investigated using a fresh lot of the medium.

Recent data on genomic mapping of E. coli have revealed that there is a definite locus of initiation of chromosomal replication irrespective of the fertility nature of the strain investigated. Both F<sup>-</sup> and Hfr strains exhibit the same replication origin.<sup>3-5</sup> The results in the present investigation indicate that there is also a definite locus for initiation of chromosomal replication in S. aureus and that the gene order is consistent among randomly selected strains of this organism.

LITERATURE CITED

1. Altenbern, R.A. 1966. Apparent genomic mapping of Staphylococcus aureus by a new method. Biochem. Biophys. Res. Commun. 25:346-353.
2. Altenbern, R.A. 1968. Chromosome mapping in Staphylococcus aureus. J. Bacteriol. 95:1642-1646.
3. Cerda-Olmedo, E.; Hanawalt, P.C.; Guerola, N. 1968. Mutagenesis of the replication point by nitrosoguanidine: Map and pattern of replication of the Escherichia coli chromosome. J. Mol. Biol. 33:705-719.
4. Helmstetter, C.E. 1968. Origin and sequence of chromosome replication in Escherichia coli B/r. J. Bacteriol. 95:1634-1641.
5. Wolf, B.; Newman, A.; Glaser, D.A. 1968. On the origin and direction of replication of the Escherichia coli K12 chromosome. J. Mol. Biol. 32:611-629.

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R & D		
(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)		
1. ORIGINATING ACTIVITY (Corporate author)		26. REPORT SECURITY CLASSIFICATION
Department of the Army Fort Detrick, Frederick, Maryland, 21701		Unclassified
		27. GROUP
2. REPORT TITLE		
A SURVEY OF GENOMIC MAPS IN STRAINS OF <u>STAPHYLOCOCCUS AUREUS</u>		
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)		
6. AUTHOR(S) (First name, middle initial, last name)		
Robert A. Altenbern		
8. REPORT DATE	72. TOTAL NO. OF PAGES	73. NO. OF REFS
March 1969	13	5
28. CONTRACT OR GRANT NO.		29. ORIGINATOR'S REPORT NUMBER(S)
A. PROJECT NO. 1B061102B71A		Technical Manuscript 520
3. 4.		30. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
10. DISTRIBUTION STATEMENT		
Qualified requesters may obtain copies of this publication from DDC. Foreign announcement and dissemination of this publication by DDC is not authorized. Release or announcement to the public is not authorized.		
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY
		Department of the Army Fort Detrick, Frederick, Maryland, 21701
13. ABSTRACT		
Six randomly selected strains of <u>Staphylococcus aureus</u> all exhibited the same gene order along the chromosome when mapped by the synchronous chromosomal replication procedure. Strains possessing multiple antibiotic resistance yielded a genomic map with all genes shifted toward significantly later replication times than in strains not bearing multiple antibiotic resistance. It appears that there is a unique site on the <u>S. aureus</u> chromosome for the initiation of replication. / )		
14. Key Words		
* <u>Staphylococcus aureus</u>		
*Genomes		
*Genetic mapping		
Genes		
Replication		
Antibiotics		

DD FORM 1473

REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Unclassified

Security Classification